

Application No. 09/754,014
Amendment Dated January 28, 2005
Revision of Reply filed 08/18/04 to Office Action of 4/20/04

Amendments to the Specification:

In response to the requested identification of amendments relative to the originally filed application rather than by numbered paragraph from the published application, identification of the placement of amendments will be in reference to the originally filed application. In addition, for the convenience of the Examiner and Applicant, as well as for the avoidance of doubt and to correct errors in US Publication No. 2002/0119940, reference is also given to the published paragraphs.

Please replace the paragraph from page 29, line 31 through page 30, line 5, which is represented by paragraph [0083] of the application as published, with the following paragraph:

[0083] The sequence of the synthetic 5' UTR (UT6) is shown below. The Kozak sequence is in **boldface and italics**, and the initiation codon is double underlined. The location of the intron (between residues 48 and 49) is indicated by the filled triangle and the sequences that form the exonic portion of consensus splice sites are single underlined. The restriction sites for HindIII and NcoI are overlined.

HindIII

NCOI

AAGCTTACTCAACACAATAACAACTTACTTACAATCTTAATTAAACAGGCCACCATG

SEQ ID NO:9

Please replace the paragraph from page 31, line 29 through page 32, line 12, which is represented by paragraph [0090] of the application as published, with the following paragraph:

[0090] The structure of the exemplary synthetic intron, OPTIVS8 is shown below (SEQ ID NO:13, with residues #1 through #9 for CAGGTAAAGT; residues #93 through #99 for TACTAAC residues #93 through #122 for TACTAACGGTCTTTTTTCTCTTCACAGG; and residues # 102 - #122 for TTCTTTTTTCTCTTCACAGG). Sequences for the 5' splice site (5'ss), branch point (bp), and 3' splice site (3'ss) are double underlined. The recognition sequences for the restriction enzymes BbsI and EarI are overlined. The cleavage site for BbsI corresponds to the 5'ss, and the cleavage site for EarI corresponds to the 3'ss. SEQ ID NO:10 residues from #1 through #15 for CAGGTAAAGTGTCTTC and SEQ ID NO:10 residues from #16 through #45 for TACTAACGGTCTTTTTTCTCTTCACAGG.

SEQ ID NO:10 (for the nucleotides shown below if depicted in a contiguous manner without random sequence ---(77)---).

5' ss bp 3' ss

| BbsI | EarI |

5' CAG GTAAGTGTCTTC---(77)---TACTAACGGTTCTTTTTTCTCTTCACAG G 3'

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Please replace the paragraph from page 32, line 13 through line 23, which is represented by paragraph [0091] of the application as published, with the following paragraph:

[0091] The 5' splice site (5'ss) sequence matches the established consensus sequence, MAG↓GTRAGT, where M = C or A, and R = G or A (SEQ ID NO: 14). Since the mechanism of splicing involves an interaction between the 5'ss of the pre-mRNA and U1 snRNA, the 5'ss sequence of OPTIVS8 (CAGGTAAGT, SEQ ID NO: 15) was chosen to be exactly complementary to the 5' end of U1 snRNA.

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5'ss          5' CAGGUAAGU 3'
              |||||
U1 RNA        3' GUCCAUUC A 5'
  
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Please replace the paragraph from page 32, line 24 through page 33, line 12, which is represented by paragraph [0092] of the application as published, with the following paragraph:

[0092] In mammals, the consensus sequence for branch points (YNYTRAY, where Y = C or T, R = A or G, N = any base, and the underlined A residue is the actual branch point, SEQ ID NO:16) is very ambiguous. Since the mechanism of splicing involves an interaction between the branch point (bp) of the pre-mRNA and U2 snRNA, the branch point sequence of OPTIVS8 (TACTAAC, SEQ ID NO:17) was chosen to maximize this interaction. (Note that the branch point itself is bulged out). The chosen sequence also matches the branch point sequence that is known to be obligatory for pre-mRNA splicing in yeast. The branch point is typically located 18 - 38 nts upstream of the 3' splice site. In OPTIVS8, the branch point is located 24 nts upstream from the 3' splice site.

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BP            5' UACUAAC 3'
              |||||
U2 RNA        3' AUGAU G 5'
  
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Please replace the paragraph from page 33, line 13 through line 24, which is represented by paragraph [0093] of the application as published, with the following paragraph:

[0093] The sequence of the 3' splice site (3'ss) matches the established consensus sequence, Y₁₁NYAG ↓ G, where Y = C or T, and N = any base (SEQ ID NO:11). In 3' splice sites, the polypyrimidine tract (Y₁₁) is the major determinant of splice site strength. For optimal splice site function in OPTIVS8, the length of the polypyrimidine tract was extended to 16 bases (Y₁₆NYAG ↓ G, SEQ ID NO:18), and its sequence was adjusted to contain 7 consecutive T residues, located in OPTIVS8 as TTCTTTTCTCTTCNYAG ↓ G, wherein Y = C or T, and N = any base (SEQ ID NO:19). This feature was included because Roscigno et al., 1993, J. Biol. Chem. 268:11222-11229, demonstrated that optimal splicing requires the presence of at least 5 consecutive T residues in the polypyrimidine tract.

Please replace the paragraph from page 33, line 25 through page 34, line 2, which is represented by paragraph [0094] of the application as published, with the following paragraph:

[0094] Splicing in vitro is generally optimal when introns are >80 nts in length (Wieringa, et al., 1984; Ulfendahl et al., 1985, Nucl. Acids Res. 13:6299-6315). Although many introns may be thousands of bases in length, most naturally occurring introns are 90-200 nt in length (Hawkins, 1988, Nucl. Acids Res. 16:9893-9908). The length of the synthetic intron (118 nts, measured from 5' splice to 3' splice, SEQ ID NO: 13) falls within this latter range.